Remarks/Arguments

The Office Action of June 2, 2003 has been carefully considered and the following response prepared. Claims 1-18 are pending in the application. Claims 1 and 12 have been amended to clarify the gene for tolerance to HPPD inhibitors and to clarify that the bleaching step is performed before transformation of the competent plant cells. Support for the amendments to claims 1 and 12 can be found throughout the specification, and in particular, at pages 3-4 and page 7, lines 33-34 and page 8, lines 1-2. No new matter has been added.

The claims of the present application are directed, in part, to methods of transforming plant cells wherein the cells are bleached with an HPPD inhibitor, then transformed with a heterologous gene and a gene for tolerance to HPPD inhibitors as a selection marker. The gene for tolerance to HPPD inhibitors comprises, in the direction of transcription, a regulatory promoter sequence which is functional in plant cells and plants, functionally linked to a DNA sequence encoding an HPPD, functionally linked to a regulatory terminator sequence which is functional in plant cells and plants.

The transformed cells are then grown and transformed cells comprising the heterologous gene and the selection marker are selected. Cells that are transformed become green when the selection marker (gene for tolerance to HPPD inhibitors) is expressed, indicating the presence in the transformed cells of the heterologous gene and the selection marker. The transformed green cells are easily distinguished from the untransformed bleached cells, facilitating the process of identifying and selecting the transformed cells. The claimed methods make it possible to decrease the time required for selecting transformed cells by several months, about 2 to 3 months. Decreasing the time for selecting transformed cells by one or more months constitutes a definite technological and economical advantage.

At page 3 of the Office Action, the Examiner rejected claims 1-18 under 35 USC 112, first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. The basis for this rejection is that "genes for tolerance to HPPD inhibitors" is not supported by the prior art and are not described in the references cited in the specification on pages 7 and 8.

Applicants again traverse this rejection. As discussed in Applicants' response to the previous Office Action, in order to satisfy the written description requirement of 112, first paragraph, the Applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the "written description" inquiry, whatever is claimed. *Vas Cath Inc. v. Mahurkur* 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). One shows that one is "in possession" of the invention by such descriptive means as words, structures, figures, diagrams, formulas, etc. that fully set forth the claimed invention. *Lockwood v. American Airlines* 41 USPQ2d 1961, 1966 (Fed. Cir. 1997).

Independent claims 1 and 12 have been amended to state that the gene for tolerance to HPPD inhibitors comprises, in the direction of transcription, a regulatory promoter sequence which is functional in plant cells and plants, functionally linked to a DNA sequence encoding an HPPD, functionally linked to a regulatory terminator sequence which is functional in plant cells and plants. Support for this amendment can be found in the specification at page 7, lines 33-34 and page 8, lines 1-2. Claims 2-11 and 13-18 which depend directly or indirectly from claims 1 and 12 are also amended by this amendment.

The amendment to claims 1 and 12 clarifies that the gene for tolerance to HPPD inhibitors is comprised of three segments, a regulatory promoter, a sequence encoding an HPPD and a regulatory terminator sequence. As disclosed at page 8 of the specification, the HPPD sequence can be a native HPPD sequence from plants, microorganisms, fungi or mammals, or a mutated HPPD sequence. Examples of several HPPD sequences are provided by reference to publications in which the sequences are disclosed. The nucleotide sequence of the W336 mutant of *Pseudomonas fluorescens* HPPD is disclosed in SEQ ID NO: 1 and SEQ ID NO: 2. Regulatory promoters useful in plants are disclosed at page 11, lines 8-30. Terminator sequences functional in plants are disclosed at page 9, lines 3-7.

As used by persons skilled in the art, a gene for tolerance or conferring tolerance to a plant disease, insect or herbicide refers to a gene or DNA sequence encoding a protein that allows a plant to resist the disease, insects or herbicide. The mode of action can be different in each instance, depending on the mechanism by which the protein encoded by the gene for/conferring tolerance acts within the plant.

Hydroxyphenylpyruvate dioxygenases (HPPD) are enzymes which catalyze the reaction in which ρ-hydroxyphenylpyruvate (HPP) is transformed in to homogentisate. Some molecules which inhibit this enzyme have been used as herbicides. Inhibition of the enzymatic reaction in plants leads to whitening of the leaves of the treated plants. In order to make plants tolerant to the HPPD inhibitors, it was found that overexpressing HPPD in plants successfully conferred on the plants agricultural level tolerance to the inhibitor. See WO96/38567 which is cited in the specification at page 8 or U.S. Patent 6,245, 968, which is the U.S. national phase of WO96/38567 cited in the specification at page 8. The same published patent application shows the use of HPPD as a selection marker in Example 6 therein.

It is not necessary, as the comments of the Examiner assert, that the HPPD itself be resistant to the HPPD inhibitors in order to confer tolerance to HPPD inhibitor type herbicides on plants. Tolerance to HPPD inhibitors can be conferred by simply overexpressing the non-mutated, sensitive enzyme. At the present time, it is believed that overexpressing non-mutated sensitive HPPD produces quantities of the target enzyme in the plant which are sufficient in relation to the herbicide, in view of the kinetic constants of the enzyme, so as to have enough of the functional enzyme available despite the presence of its inhibitor.

Applicants have shown with reasonable clarity that, as of the filing date, they were in possession of the invention. Claims 1-18 comply with the written description requirement of section 112, first paragraph. Withdrawal of this section 112, first paragraph is requested.

At page 4 of the Office Action, the Examiner rejected claims 1-18 under 35 USC 112, first paragraph as not enabled for the reasons asserted in the Office Action mailed November 7, 2002. Applicants would like to thank Examiner Kallis for Faxing a copy of the complete Chaleff et al. (1987) reference to their attorney.

In the present Office Action the Examiner stated that the teachings of the Chaleff '971 patent are broadly applicable to any method of tissue culture wherein selection for resistance to herbicides is performed on untransformed cells in the presence of an amount of herbicide that would not kill the plant tissue over the time of the exposure. Applicants again assert that the Chaleff '971 patent is not applicable to the claimed methods of transforming plants or preparing transgenic plants. The methods of the invention are not directed to selection for resistance to herbicides performed on untransformed cells. By contrast, in the claimed methods selection is performed on cells transformed with a gene for tolerance to HPPD inhibitors.

Claims 1 and 12 have been amended to insert the step of bleaching the competent cells between steps (a) and (b), now steps (a) and (c), to clarify that the cells are exposed to the HPPD inhibitor prior to transformation and selection. In the claimed methods, competent plant cells are bleached with an HPPD inhibitor and then transformed with the heterologous gene and the selection marker. Afterwards, transformed cells comprising the heterologous gene and the selection marker are grown and selected in a suitable medium. As disclosed in the specification at page 3, lines 31-34 and page 4, lines 1-3, cells that are transformed are selected by their green color which appears after transformation when the DNA sequence encoding HPPD is expressed and the HPPD is produced. Claims 1 and 12 have also been amended to state that the transformed cells appear green indicating the presence in the cells of the heterologous gene and selection marker.

In the present Office Action, the Examiner directed Applicants' attention to page 421 of Chaleff et al., and the lines directly under the heading "MUTATION BREEDING" as support for the position that the claims are not enabled:

Chaleff et al. discusses methods of developing plant varieties resistant to sulfonylurea herbicides. Page 416, second paragraph states that three different genetic strategies have been employed to modify the responses of plants to sulfonylurea herbicides: *in vitro* selection (pages 416-419), transformation (pages 419-421), and mutation breeding (pages 421-423). *In vitro* selection is discussed at pages 416-419. *In vitro* selection as described by Chaleff et al. refers to the selection of mutant herbicide resistant cells from untransformed cells grown in the presence of herbicide.

The Examiner pointed out two sentences at the beginning of the first paragraph of the section entitled "MUTATION BREEDING", which are set out below, as support for his position that the claims are not enabled:

> "The experimental advantages of genetic modification at the cellular level, either by mutant selection or transformation are not to be enjoyed without certain concessions. Passage through cell culture, which is a requirement of both techniques, imposes several severe limitations on their applicability."

The first paragraph of the section entitled "MUTATION BREEDING" discusses limitations on applicability of mutant selection (i.e., *in vitro* selection) and transformation. These limitations are (1) *in vitro* selection requires expression at the cellular level of the trait to be modified and (2) recovery in whole plants of mutant traits introduced by *in vitro* selection requires that morphogenetic capacity be retained through a sufficient number of passages in culture for selection to be effective. The paragraph ends by discussing transformation stating

> "By contrast, transformation by infection of leaf discs with Agrobacterium tumefaciens, a procedure that has been successfully applied to several dicotyledonous species (9), places far less stringent demand on regeneration."

When taken in the context of the complete publication, it can be seen that even though the sentences of Chaleff et al. pointed out by the Examiner are in the section "Mutation Breeding" the statements refer to in *vitro selection* and transformation. To the extent the asserted sentences relate to *in vitro* selection, Applicants again assert that Chaleff at al. is not relevant to the claimed methods. Selection in the claimed methods is done with transformed cells not untransformed cells. With respect to transformed cells, Applicants respectfully submit that Chaleff et al. also does not support the rejection because the paragraph itself indicates that transformation of cells and regeneration of plants "places a far less stringent demand on regeneration" and cites a successful transformation process.

It should be kept in mind that Chaleff et al. was published in 1987, and that in the thirteen years between the publication of Chaleff et al. and the application's priority date in 2000, enormous advances were made in transformation of plant cells and the regeneration of transformed plants. Even if Chaleff et al. might have arguably raised some questions about the possibility of obtaining transformed plants in 1987, this publication certainly does not represent the state of the art thirteen years later in 2000. Any concerns about culturing of transformed cells in 1987 were no longer a problem by 2000 when the priority application was filed because by 2000 regeneration of transformed plants had become well-known. The specification at page1, lines 21-26 lists numerous publications and patents disclosing methods of transforming plant cells. Methods for regenerating transformed plants were also well known in 2000. See, for example, the references on page 8 of the specification relating to DNA sequences encoding HPPD. Chaleff et al. is therefore irrelevant to the enablement of the claimed methods.

The Examiner also asserted that Applicant has not shown the presence of the transgene in any of the putatively transformed tissues of Example 2, but has only taught how to select for resistant cells. As explained below, Example 2 does indeed show the claimed methods.

In Example 2, plant callus cells are cultured in D20 medium containing 2 mg/l of isoxaflutole or 0.5 to 5 mg of diketonitrile for 10 to 15 days prior to bombardment to bleach the tissue. Bombardment refers to transformation of cells using nucleic acid coated particles that are propelled into the cells by high velocity such as by shooting from a particle gun. (See Example 1.) The cells were transformed with pCH73 or pCH94 both of which contain HPPDW336 as the selection marker, as described on pages 8 and 9 of the specification. After bombardment, the cells are placed in the same D20 medium containing 2 mg/l of isoxaflutole or 0.5 to 5 mg of diketonitrile and transferred to fresh medium every 15 days. After four transfers, green calluses are identified and amplified as described in Example 1. The presence of a green color in the callus tissue signals the presence of the selection marker in the cells. Transformed cells which have integrated the selection marker in to their genome become green, enabling them to be selected. (See pages 3 and 4 of the specification.) Table 1 shows the results of bleaching the cells before and after bombardment with pCH73 or pCH94. pCH94 showed a greater number of calluses identified when the cells were bleached prior to transformation rather than after transformation .

Example 3 also demonstrates the claimed methods. In Example 3, bombardment (i.e., transformation) of plant tissues bleached with isoxaflutol produced green calluses and required only one transfer of calluses before the selected green calluses were produced against four transfers when using a different medium.

Finally, the Examiner asserted that Applicants have not provided any guidance for identifying, isolating or evaluating the broadly claimed category of genes for tolerance to HPPD inhibitors. While the grounds for this part of the rejection are not clear, it appears that the Examiner believes genes for tolerance to HPPD inhibitors are not enabled because there are no teachings or examples showing genes that have been enhanced for increased tolerance to HPPD inhibitors and hence Applicants have not enabled methods of using a multitude of non-exemplified genes.

As explained above, the claims of the present application are directed to methods of transforming plant cells or producing transformed plants wherein a gene for tolerance to HPPD

inhibitors is used as a selection marker, not as a way to generate mutants tolerant to HPPD inhibitors. The claims have been amended to clarify the gene for tolerance to HPPD inhibitors. In the claims as amended, the gene for tolerance to HPPD inhibitors comprises, in the direction of transcription, a regulatory promoter sequence which is functional in plant cells and plants, functionally linked to a DNA sequence encoding an HPPD, functionally linked to a regulatory terminator sequence which is functional in plant cells and plants. The specification discloses examples of suitable DNA sequences encoding HPPD at page 8. Tolerance to HPPD inhibitors can be conferred by simply overexpressing HPPD in the plants or plant cells (i.e. transformed plants or plant cells containing an additional copy of non-mutated, sensitive HPPD). There is no requirement that the HPPD itself have any increased tolerance to HPPD inhibitors in order to confer tolerance to HPPD inhibitors to transformed plants or plant cells.

The Examiner has provided no reasonable basis for doubting the enablement of claims 1-18. The claims must be taken as being in compliance with section 112, first paragraph. Withdrawal of this section, 112, first paragraph rejection is requested.

In view of the above, the present application is believed to be in a condition ready for allowance. Entry of the amendments to the claims is requested as they are believed to place the application in condition for allowance or at least in better condition for appeal. Reconsideration of the application is respectfully requested and an early Notice of Allowance is earnestly solicited.

No fee is believed to be due. Please charge any fees that may be associated with the filing of this response to Deposit Account 03-2775.

Respectfully submitted,

CONNOLLY BOVE LODGE & HUTZ LLP

Date: September 3, 2003

By: _____

Liza D. Hohenschutz
Reg. No. 33,712
P.O. Box 2207
Wilmington, Delaware 19899
(302) 888-6420
Attorney for Applicants

280919